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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61K 39/12, C07K 3/00, 13/00, 15/00

A1

(11) International Publication Number: WO 94/20136

(43) International Publication Date: 15 September 1994 (15.09.94)

(21) International Application Number: PCT/US94/01847

(22) International Filing Date: 28 February 1994 (28.02.94)

(30) Priority Data:
08/027,524 8 March 1993 (08.03.93) US

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(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: CYCLODEXTRIN-PEPTIDE COMPOSITIONS

(57) Abstract

This invention provides improved compositions containing cyclodextrin complexes of peptides, particularly synthetic peptides and peptides of ≤ 40 amino acids. Such peptides are particularly useful for administration as receptor agonists, receptor antagonists, and as vaccines. The compositions of the invention provide improved means for delivery of such peptides.

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CYCLODEXTRIN-PEPTIDE COMPOSITIONS

Field of the Invention:

This invention relates to a method of presenting pharmaceutically active peptides, particularly receptor blockers and immunogenic peptides, in cyclodextrin compositions.

Background of the Invention:

Cyclodextrins are cyclic molecules containing six or more α-D-glucopyranose units linked together at the 1,4 positions. The 2-hydoxypropyl-ß-cyclodextrin (HPCD) has been used for stabilization and solubilization of various compounds, including proteins and steroids. Brewster, et al. described use of cyclodextrins in solubilizing proteins to prevent aggregation , precipitation, and loss of biopotency. ("Application of 2-hydroxypropyl beta cyclodextrin to Proteins", Minutes Int. Symp. Cyclodextrins, 5th, 1990, pp 440-444) The proteins studied therein were interleukin-3, and insulin, two large regulatory proteins. There is no suggestion that the 2-hydroxypropyl beta cyclodextrin would be useful in formulating peptides for use as receptor blockers or immunogens. The Brewster article suggests that the improved potency of the proteins is due to the avoidance of hydrolysis, deamidation, racemization, oxidation and disulfide bond exchange, and changes in dimensional protein structure related to folding of the protein. There is no suggestion that the cyclodextrins can be useful for formulations containing synthetic peptides, nor is there any suggestion that the preparations disclosed therein can be administered by application to the mucosa.

Josef Pitha, in U.S. patent 4,727,064, which is incorporated herein by reference, suggests the use of cyclodextrin in solubilizing medicinals including steroids and vitamins, but does not disclose the solubilization of peptides in cyclodextrin.

Szejtli, et al., in U.S. patent 4,380,626, teach the use

of cyclodextrins in preparations of plant growth regulators including 2-chloroethylphosphonic acid. No use of cyclodextrins for preparation of peptides is taught or suggested therein.

Gideon Goldstein, in U.S. Patent 5,140,010, which is incorporated herein by reference, teaches the stabilization of aqueous formulations of synthetic peptides corresponding to position 32-36 of thymopoietin and known as thymopentin or TP-5 (Arg-Lys-Asp-Val-Tyr) in glycine. Goldstein does not disclose or suggest use of cyclodextrin for stabilization of peptides. TP-5 is effective in blocking the stimulation of smooth muscle contraction caused by the neurotoxin (+)-anatoxin-a (ANTX). ANTX is a bicyclic amine exotoxin produced by the blue-green algae, Anabaena flos-aquae, and has been found to cause death to livestock and waterfowl. The toxin acts by depolarizing blockade of neuromuscular transmission. Such depolarization results in respiratory paralysis. The action of ANTX has been ascribed to its potent nicotinic cholinergic agonist activities in skeletal muscle and mammalian skeletal muscle and the central nervous system. ANTX can also cause cardiovascular aberrations by activation of nicotinic receptors in the adrenal medulla and sympathetic ganglia. The antagonist effect of TP-5 has been attributed to its ability to block nicotinic receptors in a noncompetitive manner.

Summary of the Invention:

This invention provides improved compositions containing cyclodextrin complexes of peptides, particularly synthetic peptides and peptides of $\leq$ 40 amino acids. Such peptides are particularly useful for administration as receptor agonists, receptor antagonists, and as vaccines. The compositions of the invention provide improved means for delivery of such peptides.

Many peptides, especially peptides of about three to 20 amino acids, are unstable in low concentrations and tend to loose biological activity. While Brewster describes the value of preparing formulations of cyclodextrin and r gulatory proteins to avoid conformational changes, there is no suggestion therein that cyclodextrin would be useful for increasing

stability of small peptides such as thymopentin.

Th instant invention improves methods of administration of peptides to the mucosa of mammals in need of treatment with effective peptides.

Detailed Description of the Invention:

The invention provides a means of formulating peptides to avoid loss of efficacy and to facilitate delivery of the active peptides to the reactive site. The method has been exemplified using the synthetic peptides corresponding position 32-36 of thymopoietin and known as TP-5 (Arg-Lys-Asp-Val-Tyr). While the hydroxypropyl cyclodextrin has been exemplified, other cyclodextrins, including mixed cyclodextrins, may be used in the method of the invention.

One problem in use of peptides is their instability in aqueous solution, especially very dilute compositions. Furthermore, many of the solvents used to provide stable, soluble compositions for treatment of other mammals can not be used in man. At present, there is no known compatible solvent for TP-5 in which the peptide is stable and easily administered. This is a significant problem because the instability will hinder acceptance for prophylactic and/or therapeutic applications.

Materials and Methods:

The 2-hydroxypropyl-β-cyclodextrin used in the examples was purchased from Pharmatec in Alachua, Florida. TP-5 (10^{-2} M) was synthesized as describe in Chiang, et al, Life Sci 49: (1991) PL13-19 and was made up in various percentages of HPCD dissolved in sterile water. Mixtures were stirred for about one hour. The solutions were then maintained at room temperature. Control solutions of TP-5 dissolved in sterile water without HPCD were also prepared in the same manner. The two sets of solutions were stored at ambient room temperatures (25°C) for 14 months. Aliquots were removed monthly for stability testing. The stability study was performed by assaying the ability of the TP-5 solutions to counteract the stimulation of contraction of guinea pig ileum by ANTX. Guinea pig ileum contraction stimulated by ANTX was performed as

reported in Chiang, et al. (supra). The final concentration of TP-5 for use was obtained by diluting with Krebs-Ringer buffer.

EXAMPLE I

Aqueous solution of 2-hydroxy-ß-cyclodextrin (HPCD) were prepared at concentration of 2.5%, 5.0%, 10%, 15%, 20%, 25% and 30% (w/v). TP-5 was added in sufficient amounts to provide a final molarity of 10^{-2} molar solution of TP-5. Further dilution to provide final dosage was made using Krebs-Ringer buffer. The solutions were then stored at ambient temperature for 14 months, after which activity of the cyclodextrin solutions was compared to freshly made solutions. As a control a 10^{-2} solution without cyclodextrin was prepared. After storage at ambient temperature (25°C) for four weeks the control solution showed no activity.

EXAMPLE II

Evaluation of Anatoxin-A Response alone and in conjunction with TP-5 was carried out in accord with standard procedures as disclosed in U.S. Patent 4,973,734 issued November 27, 1990, which is incorporated herein by reference.

Results:

IC_{50} values of the inhibition by TP-5 of guinea-pig ileum contraction stimulation by ANTX at 3 x 10^{-5} N was compared using freshly made 10_{-2} molar solutions of TP-5 and similar concentrations of TP-5 in 5%, 15% and 20% solutions of HPCD which had been stored for 14 months at ambient temperature to determine relative activity. The results are shown as mean ± s.e. of four separate experiments as indicated in Table I

TABLE I

HPCD (%)	IC_{50} (x 10^{-5} M)
0 (freshly made)	3.9 ± 1.9
5%	4.1 ± 3.1
15%	4.9 ± 4.4
20%	3.3 ± 3.0

EXAMPLE III

A composition containing 0.5 mg TP-5 is administered intraperitoneally to rabbits to provide protection against ANTX. Formulations may be administered for up to 4 days. Dosage range for thymopentin may vary from 1 μg/kg/day to 1 g/kg/day. It is, of course, understood that smaller animals will require higher dosage per kilogram than larger mammals.

Formulations of active agents in HPCD for administration may be prepared using any pharmaceutically appropriate solvent, including water, isotonic saline, glucose, or saline. The formulations may be administered orally in the form of liquid bolus, or may be administered as lyophilized powders or tablets. When provided as lyophilized powders, many of the compositions may be administered nasally for inhalation. Compositions of the invention may be administered parenterally by, for example intramuscular, subcutaneous or intraperitoneal routes. Solutions of the cyclodextrin inclusion complexes can be administered to the mucosa by any means appropriate such as by nasal spray, buccal tablet or sublingually as drops. The site of administration will be governed, in many instances, by the site of effective response. For example, it is often advantageous to administer immunogenic peptides to the mucosa.

Many other peptides could be formulated in a similar manner. Such peptides include splenopentin (SP-5) having the structure Arg-Lys-Glu-Val-Tyr. This peptide is effective for inducing T-cell differentiation and for modulation of neuromuscular transmission. (Proc. Natl. Acad. Sci. USA 81: 2847-2847 (1984)) Others include a nine amino acid sequence known as delta sleep inducing peptide (DSIP) of the structure Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu (Neurosci. Biobehav. Rev. : 83-93 (1984)), vasoactive intestinal peptide (VIP) or biotinyl-VIP from human, porcin, chick, rat or other sources, having the sequence His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2 for prevention for cell killing by human immunodeficiency virus (Nature 335: 639-642 (1984)) and for pharmacological treatment of tissues involving n uromuscular transmission (Arch. int.

Pharmacodyn 305, 14-24 (1990)). The peptide HG165-178 representing the sequence 165-178 of gp120 is represented by the sequence Asn-Ile-Ser-Thr-Ser-Ile-Arg-Gly-Lys-Val-Gln-Lys-Gln-Lys-Glu-Tyr, which is analogous to sequences in snake neurotoxins and rabies virus glycoprotein is conjugated to a keyhole limpet hemocyanin (KLH) and can be, thereafter, encapsulated in cyclodextrin to prevent the binding of viruses, toxins, viral coatings and gp120 to cells. (See FEBS Letters 311: 115-118 (1992)).

The methods of the invention should be particularly considered to stabilize peptides containing asparytyl, asparaginyl and glycine residues.

CLAIMS

1. A pharmaceutically effective composition comprising an effective amount of a receptor agonist, antagonist or immunogenic peptide of 3 to 40 amino acids in a cyclodextrin inclusion complex in a pharmaceutically acceptable diluent.

2. A composition of claim 1 wherein the immunogenic peptide is TP-5.

3. A composition of claim 1 wherein cyclodextrin is present at a concentration of .5% to 30%.

4 A composition of claim 3 wherein a cyclodextrin is 2-hydroxypropyl-ß-cyclodextrin.

5. A composition of claim 1 wherein the active peptide is an immunogen.

6. A composition of claim 1 wherein the peptide is splenopentin.

7. A composition of claim 1 wherein the peptide is delta sleep-inducing peptide.

8. A composition of claim 1 wherein the peptide is vasoactive intestinal peptide.

9. A composition of claim 1 wherein the peptide is HG 165-178.

10. A method of administering an immunogen to an animal by administering an immunogenic effective amount f a pharmaceutical composition of claim 5.

11. A method of claim 10 wherein the pharmaceutical composition is administered directly to the mucosa.

12. A method of claim 11 wherein the pharmaceutical composition is administered sublingually.

13. A method of claim 11 wherein the pharmaceutical composition is administered to the nasal mucosa by inhalation.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01847

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 39/12; C07K 3/00, 13/00, 15/00
US CL : 424/89, 85.1, 88,; 530/395, 350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/89, 85.1, 88,; 530/395, 350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, search terms: cyclodextrin, pharmaceutical, thymopentin, splenopentin, vasoactive intestinal peptide, neuronal peptide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,923,964 (GOLDSTEIN ET AL) 08 May 1990, cols. 7-10.	1-13
Y	US, A, 5,140,010 (GOLDSTEIN ET AL) 18 August 1992, see entire patent.	1-13
Y	US, A, 5,024,998 (BODOR) 18 June 1991, cols. 1-11.	1-13
Y	US, A, 4,956,274 (KHANNA ET AL) 11 September 1990, cols. 9-12.	1-13
Y	Nature, Volume 335, issued 13 October 1988, D. E. Brenneman, et al, "Neuronal Cell Killing by the Envelope Protein of HIV and its Prevention by Vasoactive Intestinal Peptide", pages 639-642, see entire article.	1-13

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proceedings National Academy Sciences, USA, Volume 81, issued May 1984, T. Audhya et al, "Contrasting Biological Activities of Thymopentin and Splenin, Two Closely related Polypeptide Products of Thymus and Spleen", pages 2847-2849, see entire article.	1-13
Y	FEBS Letters, Volume 311, No. 2, issued October 1992, L. Bracci, et al, "Binding of HIV-1 gp120 to the Nicotinic Receptor", pages 115-118, see entire article.	1-13